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Research Article

Histological Evaluation of Peri-Implant Soft Tissue Response to Direct Contact with Titanium Screw Surface in a Rabbit Model

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Abstract: Rabbits present biological features that make them suitable for morphological and histological investigations that allow a closer approximation to human tissue responses, particularly with respect to immune and vascular reactions. Titanium screws measuring 5 mm in length and 2 mm in diameter were inserted into the femoral bone of five 14-month-old female rabbits of a common breed, one on the right side and one on the left. Femoral segments containing the implants were subsequently harvested, fixed in 10% formalin, decalcified with trichloroacetic acid, and embedded in paraffin. Sections 5 µm thick were cut, stained using the Goldner trichrome method, and microscopically examined. At the interface between the surface of the screw head and the newly formed tissues, a separating capsule was formed through condensation of the connective tissue. Within the medullary cavity, the bone marrow that encountered the screw surface responded by gradually regenerating with tissue of the same type, and did not exhibit a significant foreign body reaction, demonstrating a remarkable tolerance toward titanium implants. This tolerance allowed peri-implant tissues to undergo repair through the formation of tissues like those present prior to surgery. The distance between the implant surface and the surrounding tissue elements did not represent a critical factor, whereas repetitive tissue movements triggered a protective response manifested by the formation of a separating capsule between the surface of the implant head and the adjacent structures.

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1. Introduction

Rabbits present biological features that make them suitable for morphological and histological investigations. The insertion of titanium implants into cortical bone in female rabbits is accompanied by injury to all tissues in the intervention area. These tissues include the bone in which the drilling site is created, as well as the supraperiosteal soft tissues and those within the medullary cavity, namely the bone marrow. After closure of the surgical wound, the implant surface is in direct contact with all these tissues. The process of implant osseointegration within bone closely resembles the one of fracture healing. For fractures to heal under normal conditions, the fractured ends must be stabilized in such a way that the distance between them is as small as possible. It is also necessary that there is no movement beyond a certain limit between the bone ends, as excessive motion may lead to the formation of a nonunion – pseudarthrosis [1]. This situation also applies to implants, where neither the gap nor the micromotion between components should exceed 150 µm; otherwise, there is a risk of connective tissue proliferation instead of bone formation [2].

These considerations apply to the relationship between the surface of the titanium implant and the surface of the insertion site wall, where the distances can be controlled by the surgical procedure used for implant placement. The situation is different in the case of contact between implant regions that do not come into direct contact with the bone wall but instead interface with soft tissues. In this case, the only fixed surface is that of the implant, whereas the soft tissues lack a stable shape, and their structural elements may be located at small, large, or even very large distances from the implant surface. This raises the question of whether these distances influence reparative processes in such a way that, around the implant head and the portion that penetrates the endosteum and enters the bone marrow, connective scar tissue is formed.

The aim of the study was to evaluate the tissues proliferating in the immediate vicinity of the screw head, which encounters supraperiosteal soft tissues, and of the implant portion that penetrates the bone marrow.

2. Materials and Methods

This study was conducted in strict accordance with the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The research protocol with animal experimentation was approved by the Scientific Ethics Committee of University of Agricultural Sciences and Veterinary Medicine (Protocol Number: 219 of 10.07.2020). All surgery was performed under anesthesia, and every effort was made to minimize suffering. Anesthesia was achieved by intramuscular administration of xylazine (5 mg/kg) and ketamine (40 mg/kg).

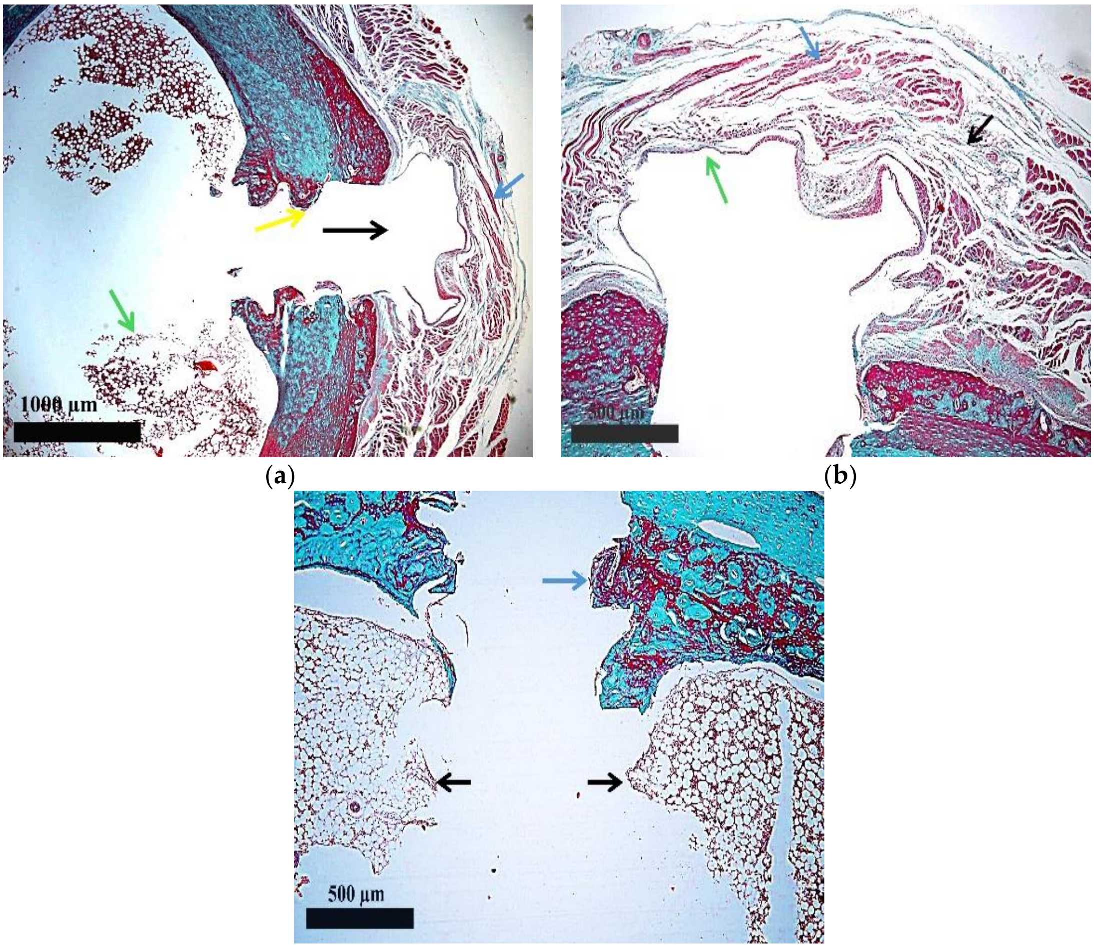
Titanium screws measuring 5 mm in length and 2 mm in diameter were inserted into the femoral bone of five 14-month-old female rabbits of a common breed, one on the right side and one on the left. Preparation of the surgical site consisted of clipping and disinfection with iodine tincture. followed by an incision of the skin and underlying tissues to expose the femur. The insertion site was drilled using a 1.8-mm diameter drill bit, with continuous cooling of the area using physiological saline. The screws were inserted in such a manner that they penetrated the endosteum and entered the medullary cavity to an appreciable extent. After routine closure of the surgical wound, Enroxil 5% (enrofloxacin) was administered subcutaneously at a dose of 20 mg/kg for 5 days as antimicrobial prophylaxis, and Meloxicam was given as an analgesic at a dose of 1 mg/kg subcutaneously for 3 days. In this experimental study, death constituted the predefined experimental endpoint. Animals were monitored daily throughout the study period for general health status, behavior, food and water intake, locomotion, and surgical site condition. No animals reached criteria requiring premature euthanasia. At the end of the two-month experimental period, all animals were humanely euthanized by a veterinarian by using a method in accordance with the provisions of EU Directive 2010/63/EU on the protection of animals used for scientific purposes (administration of an overdose of sodium phenobarbital). Death was confirmed prior to tissue collection by the absence of heartbeat/respiration and corneal reflex.

Femoral segments containing the implants were subsequently harvested, fixed in 10% formalin, decalcified with trichloroacetic acid, and embedded in paraffin. Sections 5 μm thick were cut, stained using the Goldner trichrome method, and examined under an Olympus BX41 microscope.

3. Results

Histological processing allowed the preparation of sections encompassing the entire surgical area, enabling assessment of all components in contact with the implant, including both hard osseous tissues and soft tissues located supraperiosteally as well as those within the medullary canal (Fig. 1a).

It was observed that the supraperiosteal area was repaired by newly proliferated tissues, consisting of loose connective tissue in which muscle fiber bundles were evident. At the interface between the surface of the screw head and the newly formed tissues, a separating capsule was formed through condensation of the connective tissue (Fig. 1b). Within the medullary cavity, the bone marrow that met the screw surface did not exhibit a significant reaction, in the sense that there was no tendency toward connective tissue proliferation. This demonstrates that the presence of the titanium implant was well tolerated by the bone marrow (Fig. 1c).



(**a**) (**b**) (**c**)

Figure 1. (**a**) Surgical site: black arrow—implant location; blue arrow—supraperiosteal soft tissues; green arrow—bone marrow; yellow arrow—bone–implant interface (Goldner trichrome, 2× objective); (**b**) Supraperiosteal soft tissues: black arrow—loose connective tissue; blue arrow—muscle tissue; green arrow—connective tissue capsule (Goldner trichrome, 4x objective); (**c**) Intramedullary soft tissues: black arrow—bone marrow; blue arrow—bone–implant interface (Goldner trichrome, 4× objective).

4. Discussion

The proliferation of reparative tissues following the insertion of a titanium implant is a complex process that differs in duration depending on the type of tissue involved. The repair of bone tissue at the bone–implant interface takes the longest, whereas peri-implant soft tissues regenerate over a significantly shorter period. Most available information concerns the evolution of reparative processes at the bone–implant interface, as this region ensures secondary stability of the implants and their long-term persistence in the body. It is known that the distance between the implant surface and the bony wall of the insertion site should not exceed a certain limit, since at greater distances connective tissue will proliferate instead of the desired bone tissue [2]. Along this portion of the interface, the distances between components can be planned and achieved through the surgical technique employed, so that from this perspective the conditions are under control. Such control cannot be exerted at the interface between the implant surface and soft tissues, because in this case only the implant surface is fixed and rigid. In relation to it, the components of the soft tissues are located at varying distances, and some of them may undergo displacement within certain limits, either relative to each other or to the implant surface. This mainly refers to the soft tissues covering the surface of the implant head. Repair of these tissues occurred in a natural manner, with loose connective tissue containing bundles of muscle fibers.

It should be noted that this loose connective tissue showed no tendency toward fibrous consolidation, which demonstrates that tissue repair in this area was not negatively influenced by the presence of the titanium implant. From this perspective, it can be stated that the peri-implant soft tissues organism tolerated the titanium implant very well. The current results are consistent with other experiments conducted on rabbits [3-11].

However, a separating connective tissue capsule was formed between the surface of the screw head and the tissues covering it. It can be considered that this capsule did not develop as a reaction of the organism to the material from which the implant was made, nor because of the distance between the surface of the implant head and the components of the adjacent loose connective tissue. Rather, its appearance seemed to be a consequence of the fact that the tissue covering the implant head undergoes movement due to contraction of the neighboring muscle tissue. These movements generated a certain degree of friction between the components of the connective tissue and the hard surface of the implant, and the organism responded by organizing this separating capsule between the loose tissue and the implant surface. By covering the implant head, this capsule protected the loose connective tissue so that it did not experience direct contact with the hard implant surface during the relatively frequent movements to which it was subjected.

Within the medullary cavity, the situation was also particular, in that the bone marrow showed no signs of injury nor proliferation of undesirable tissues, such as connective tissue. In other words, although the marrow was traumatized to a certain depth during the procedure required for implant insertion, it responded by gradually regenerating with tissue of the same type, namely bone marrow. This demonstrates that the marrow did not react to the presence of the implant as to a foreign body; on the contrary, it exhibited a remarkable tolerance toward it. Moreover, the distance between the implant surface and the components of the marrow was greater than that between the implant surface and the bony wall, and even greater than that between the implant surface and the constituent elements of the connective tissue covering the implant head. Nevertheless, this distance did not constitute a major problem capable of inducing the formation of separating structures, as observed in the case of the supraperiosteal tissues covering the screw head. It can therefore be stated that the distance between components did not significantly influence reparative processes in the case of all tissues adjacent to the surface of titanium screws.

When the peri-implant soft tissues were comparatively evaluated in terms of their response to proximity with the surface of titanium screws, it was evident that they tolerated this relationship very well. In this context, the injured tissues were repaired by the same tissue types that had been present in the area prior to the surgical intervention. Nevertheless, a separating capsule developed between the implant head and the newly proliferated tissues, whereas no such structure was observed between the bone marrow and the implant.

This formation did not appear to represent a rejection response to the implant material, nor was it related to the distance between the screw surface and the surrounding tissue elements. Instead, it appeared to have an objective cause specific to the anatomical region involved. A distinctive feature of the tissues covering the implant head was their composition, consisting of loose connective tissue containing bundles of muscle fibers. Through contraction of these muscles, the structural elements of the loose connective tissue underwent displacement within certain limits. Consequently, these tissues exhibited repetitive mobility, which subjected those in direct contact with the implant surface to friction against the rigid surface of the implant. This friction was perceived as a repetitive mechanical aggression, leading to the organization of a protective separating capsule that eliminated direct friction between the adjacent tissues and the implant surface.

5. Conclusions

Most of the studies focus on osseointegration and hard tissue responses to titanium implants. This research emphasizes the biological behavior of soft tissues at the implant interface, offering detailed microscopic evidence of tissue organization. In conclusion, supraperiosteal and medullary soft tissues exhibited a high degree of tolerance toward titanium implants, without evidence of foreign body reactions. This tolerance allowed peri-implant tissues to undergo repair through the formation of tissues like those present prior to surgery. The distance between the implant surface and the surrounding tissue elements did not represent a critical factor, whereas repetitive tissue movements triggered a protective response manifested by the formation of a separating capsule between the surface of the implant head and the adjacent structures. The current study is relevant for implant dentistry and biomaterials, as it supports the optimization of implant surface design and surgical protocols aimed at improving long-term clinical outcomes and reducing peri-implant complications.

Author Contributions:

Conceptualization: A.D., M.I.C.; Methodology: B.F., A.D., F.G.S.; Investigation: B.F., D.P., C.-R.C.; Histological analysis: B.F., I.I.; Writing – original draft: B.F., M.I.C.; Writing – review and editing: M.I.C., I.I.; Supervision: A.D., M.I.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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